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GLYCOGEN IN THE CHICK EMBRYO.

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In his paper, "Glycogen in the Nervous System of Vertebrates"¹ Gage has pointed out that glycogen is not found in such great abundance in the organs of the chick at any one period of its development as in the embryonic organs of many other forms, including mammals; from which the inference is at once drawn that this difference is correlated with the nature of development in the hen's egg, where the yolk furnishes an abundant supply of food, available at any moment for the needs of the embryo. The development of the various organs is a gradual one as compared with that of forms which are provided with less yolk, and glycogen, the builder of tissue and energy producer, appears only as it is needed in the successive stages.

This is particularly striking when comparing the chick with such forms as *Petromyzon* and *Amblystoma*, studied by Gage. When contrasted with mammals the same explanation is not as valid, for the mammalian tissue is also able to develop gradually, calling upon the maternal supply when necessary. However it may be supposed that in mammals the embryo is less directly in contact with its food supply than in the chicken. A great amount of glycogen could not be produced as quickly when a demand arose in tissue development, and consequently a greater supply is produced and maintained in the embryonic tissue itself.

The following observations in regard to the occurrence and distribution of glycogen in the tissue of the chick, from primitive streak stages to the age of about ten days, are based on material which was fixed and stained according to the methods given in Gage's article, and in all cases the presence of the glycogen has been verified by testing the sections with saliva, according to Gage's directions.

It gives me pleasure to be able to acknowledge my great

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indebtedness to Dr. H. L. Wieman, under whose direction this problem was undertaken, and whose assistance, interest and criticism made it possible to carry out the work.

Perhaps the most striking fact observed in the formation of glycogen in the chick was its almost invariable presence in the yolk-sac, from the earliest stages to chicks of ten days. Fig. 1 (Plate I.) shows globules of glycogen in the yolk-sac of a chick of six somites, before the vascular area has developed. In these earlier stages the glycogen is located in the yolk-sac near the embryo, and it is found more or less consistently distributed throughout the vascular area when this has been formed, more abundant near the embryo than towards the edges. It gives to the walls of the blood-vessels of this region a striking red color and under high power appears as mahogany-colored masses scattered among the cells. Fig. 2 shows a part of the vascular area of a six-day chick with the glycogenated areas represented in stippling in the walls of the blood-vessels. Figs. 2*a* and 2*b* show parts of the vascular areas of seventy-two-hour and four-day chicks respectively, under higher magnification. In Fig. 2*b* the actual globules of glycogen are represented in black. Apparently the yolk-sac furnishes a way-station in which carbohydrates are stored as glycogen on their way from the yolk to the embryonic tissue.

This formation of glycogen in the yolk-sac makes an interesting comparison with the formation of glycogen in the placenta of mammals, a fact which seems well established. In mammals the maternal glycogen-forming tissues are perhaps relieved in this way of the strain of a sudden call for large amounts of sugar. In both cases a supply of glycogen is on hand ready-formed and able to be called upon quickly.

In the earliest chick embryos observed there appeared to be, here and there, a trace of glycogen in the yolk itself in the form of globules somewhere near the site of the embryo, but this fact is not at present established beyond a doubt in my mind, although it would accord with what Gage has observed in the eggs of the *Petromyzon* and *Amblystoma*.

In embryos of from fifteen to twenty-four hours glycogen was found in the ectoderm of the head region, both in the developing

nervous system and in the ectoderm below the headfold (Fig. 3).

As soon as the heart tissue begins to develop (at about twenty-eight hours) it takes on its glycogenic function and from that time to the oldest embryos studied the heart is the most consistently and by far the most abundantly glycogenated organ of the body. The glycogen here appears in very deeply stained granules embedded close together throughout the muscular walls of the entire heart, and extending into the sinus venosus and the bulbus. Fig. 4 shows, in stippling, the position of the glycogen in the heart tubes of a chick of nine somites. Fig. 5 shows a part of the heart of a six-day chick, with glycogen abundant in the muscles. Fig. 5*a* represents a part of the wall of a four-day chick's heart, and Fig. 5*b* of a ten-day chick's heart, under high power. In both of these the actual globules of glycogen have been represented in black.

The glycogen of the vascular area seems to be continued under the embryo with more or less regularity. Certainly as the gut is formed glycogen extends into it, appearing in some cases very distinctly at the opening of the gut onto the yolk, more abundant at the opening of the hind-gut than of the foregut, extending in a thirty-five-hour chick into the pharynx, and from fifty-six hours on being very noticeable in the intestine. Fig. 6 shows the fore-gut of a four-and-a-half-day chick just in front of the anterior portal. The glycogen in the entoderm of the gut and below it is stippled. Fig. 7 is the posterior intestinal portal of a seven-day chick, and Fig. 7*a* is the intestine and splanchnic entoderm of the same a few sections posterior to Fig. 7. Fig. 8 is a transverse section through the intestine of a ten-day chick, showing glycogen abundant in the epithelium.

At fifty-six hours, and in all later stages, glycogen was found in the myotomes, being particularly striking in those sections where the body was cut sagittally, the glycogen here appearing like mahogany-colored beads on the spindle-shaped myotomes (Fig. 10, Plate II.). Fig. 9 (Plate II.) is a transverse section of a chick of five days showing the position of the glycogen in the myotomes. Under high power one of these myotomes appeared as in Fig. 11, where the actual spots of glycogen are represented. As other muscular parts develop the myotomes become less

glycogenated, but they still retain this function to some extent.

In a seventy-two-hour chick there was an appearance of glycogen in the mesoderm between the two portals and the two sides of the future body-cavity, but as this did not seem consistent with other stages, its presence, or the normality of its presence, is rather doubtful.

In a ninety-six-hour chick a faint trace of glycogen was found in the septum medullæ. At four and a half days the glycogen was very distinct here and it remained in this position up to the oldest stage studied, making a very striking mahogany-colored streak along the floor of the medulla (Figs. 12 and 13). From five days on it was found in a similar position along the floor of the spinal cord in its lumbar and sacral regions (Fig. 14).

At this same age (five days), it appeared in the ectoderm of the anal plate.

At approximately six days glycogen appeared in some of the muscular tissue around the eyes, forming strings of beads along the fibers of the tissue. This became more and more abundant in later stages (Figs. 15 and 16).

At an age of from seven to eight days it was observed in other parts of the head region—in the muscular tissue below the tongue, and medially in a clearly defined area of developing cartilage which formed a keel-like structure below the brain and between it and the pharynx. (Fig. 17).

At this age also glycogen occurs in the muscular tissue along the sides of the body, and at a slightly older stage it appears in a small patch of ectoderm in the nose region.

At ten days it is found in the cartilage of the head and body quite generally. Fig. 18 shows a trace of it in a developing centrum in the head region. In Fig. 14 it appears in the vertebral cartilage of the body. Fig. 19 shows it very abundant in the cartilage of one of the developing limbs. Fig. 19*a* shows the same under high power. The glycogen of the cartilage appears as very distinct red spherules which practically fill the cells.

Glycogen is found in great abundance at this age in the muscular tissue, not only in that already mentioned, but also in the more superficial muscles of the head, in the pectoral muscles (Fig. 20), in the muscles of the limbs (Fig. 19), and in fact in

practically all of the musculature of the body. In some places it seemed to occur in the deeper layer of the skin.

It appeared at this age also in a mass of nerve cells lying in the dorsal fissure of the spinal cord in its lumbar region (Fig. 14). Its occurrence in these cells was also noted by Gage.

These results are perhaps more significant in a negative than a positive way. They show that in the chick the storage of glycogen in the embryonic tissue itself is not as extensive as in other forms, and that it does not become a well-developed function of the embryo in its earliest stages, though glycogen does appear in some of the tissue and becomes more and more abundant in later stages. The embryo depends for its energy production and building material upon the food of the yolk, a part of which is kept on hand in the form of glycogen in the yolk-sac.

The majority of the drawings were made with a 16 mm. objective and 4x eye-piece. For those marked high power a 4 mm. objective, 4x eye-piece were used. All drawings were made with a camera lucida.

EXPLANATION OF PLATE I.

FIG. 1. Yolk-sac of a six-somite chick before development of the vascular area. Glycogenated cells in black.

FIG. 2. Splanchnopleure of the vascular area of a six-day chick with glycogen in the walls of the blood-vessels (stippled). *bl. v.*, blood-vessels; *cæl.*, cœlome; *y.s.*, yolk-sac.

FIG. 2*a*. Vascular area of a seventy-two-hour chick under high power. (Glycogen stippled.)

FIG. 2*b*. Vascular area of a four-day chick, high power. Actual masses of glycogen represented in black.

FIG. 3. Glycogen (stippled) in the ectoderm of a chick with six somites. *ect.*, ectoderm.

FIG. 4. Glycogen (stippled) in the heart tubes of a chick with nine somites. *ht.*, heart; *ph.*, pharynx.

FIG. 5. Heart of a six-day chick with glycogen abundant in the muscular walls. (Stippled.) *l.a.*, left auricle; *l.v.*, left ventricle; *r.a.*, right auricle; *r.v.*, right ventricle.

FIG. 5*a*. Wall of a four-day chick's heart under high power. The black portions represent glycogen.

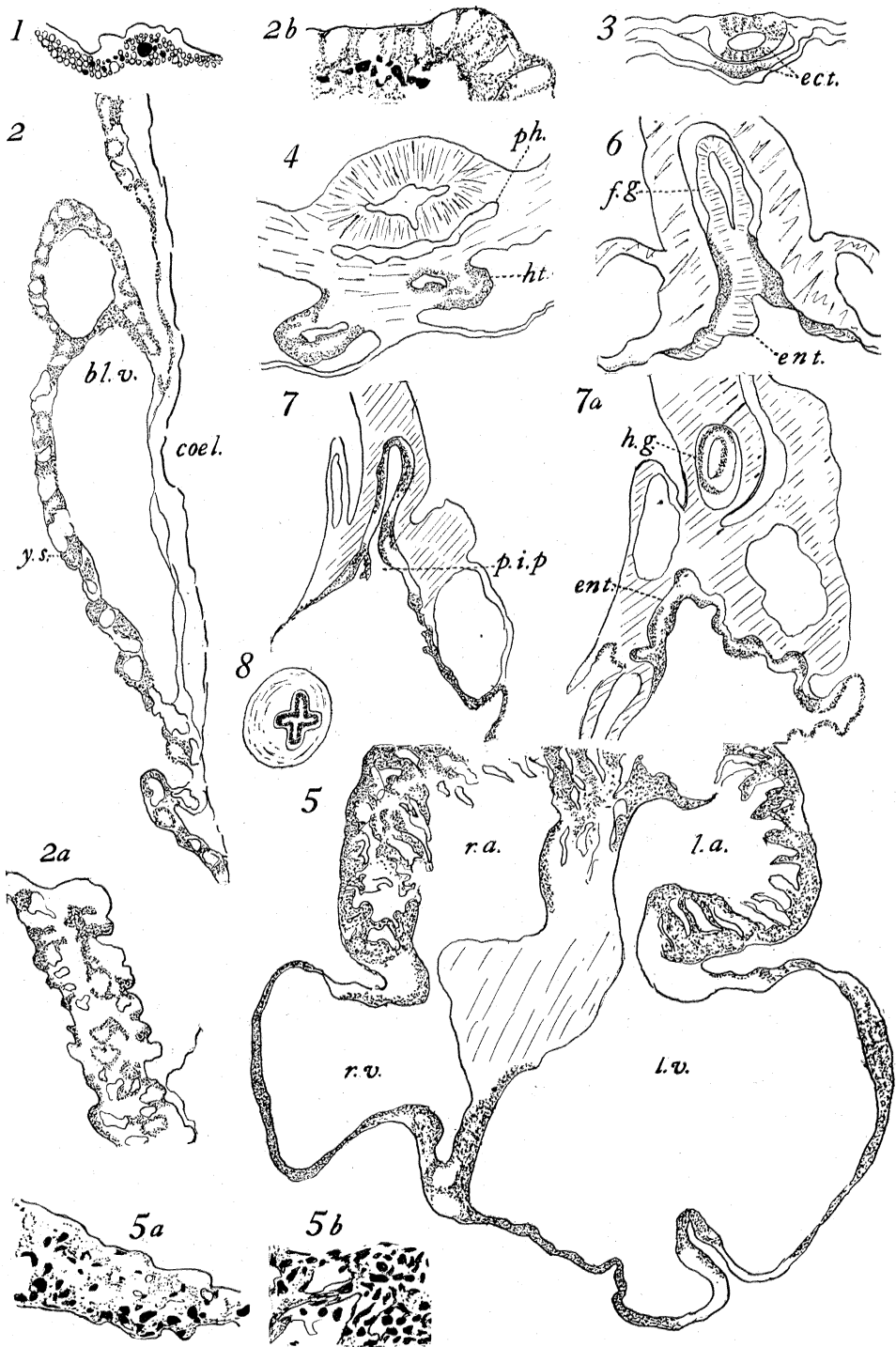
FIG. 5*b*. Ten-day chick's heart. High power. Globules of glycogen in black.

FIG. 6. Fore-gut of a four-and-a-half-day chick just anterior to the anterior portal. (Glycogen stippled.) *ent.*, entoderm; *f. g.*, fore-gut.

FIG. 7. Posterior intestinal portal of a seven-day chick. (Glycogen stippled.) *p.i.p.*, posterior intestinal portal.

FIG. 7*a*. Hind-gut and splanchnic entoderm of the same, a few sections posterior to 7. (Glycogen stippled.) *ent.*, entoderm; *h.g.*, hind-gut.

FIG. 8. Cross section through intestine of a ten-day chick. (Glycogen stippled.)



EXPLANATION OF PLATE II.

FIG. 9. Transverse section of a five-day chick showing glycogen (stippled) in the myotomes and intestine. *m.y.*, myotomes; *int.*, intestine

FIG. 10. Sagittal section through the posterior end of a seven-day chick showing glycogen (stippled and dotted) in the myotomes. *m.y.*, myotomes.

FIG. 11. A myotome in sagittal section. Five days. High power. The black areas are glycogen.

FIG. 12. Transverse section of the medulla oblongata of a six-day chick. (Glycogen stippled.) *m.*, medulla; *s.m.*, septum medullæ.

FIG. 13. Sagittal section of the septum medullæ. Six to seven days. (Glycogen stippled.) *c.*, cord, *s.m.*, septum medullæ.

FIG. 14. Transverse section of spinal cord (lumbar region) of a ten-day chick, showing glycogen (stippled) in the cartilage of the vertebra, the floor of the cord and a mass of nerve cells in the dorsal fissure. *ca.*, cartilage; *n.*, glycogenated nerve tissue.

FIG. 15. Glycogenated muscle near the eye of a six-day chick. (Glycogen stippled.) *dien.*, diencephalon; *no.*, optic nerve; *o.*, eye.

FIG. 16. Glycogenated muscles near the eyes of a seven-day chick. (Abbreviations as in 15.)

FIG. 17. Glycogenated cartilage of developing skull. Seven to eight days *ca.*, cartilage; *dien.*, diencephalon; *o.*, eye; *ph.*, pharynx.

FIG. 18. Developing centrum in the head of a ten-day chick, showing glycogenated area in stippling. *ca.*, cartilage.

FIG. 19. Glycogenated cartilage and muscles of the foot of a ten-day chick. (Glycogen stippled and dotted.) *ca.*, cartilage; *m.*, muscles.

FIG. 19a. Cartilage of the same under high power.

FIG. 20. Pectoral muscles and cartilage of a ten-day chick.

